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BY

T. M. TIMONEY, M.R.C.V.S.,
Third Bacteriologist, Muktesar.

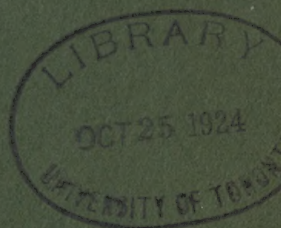


(Reprinted from the Proceedings of the Fifth
Entomological Meeting)

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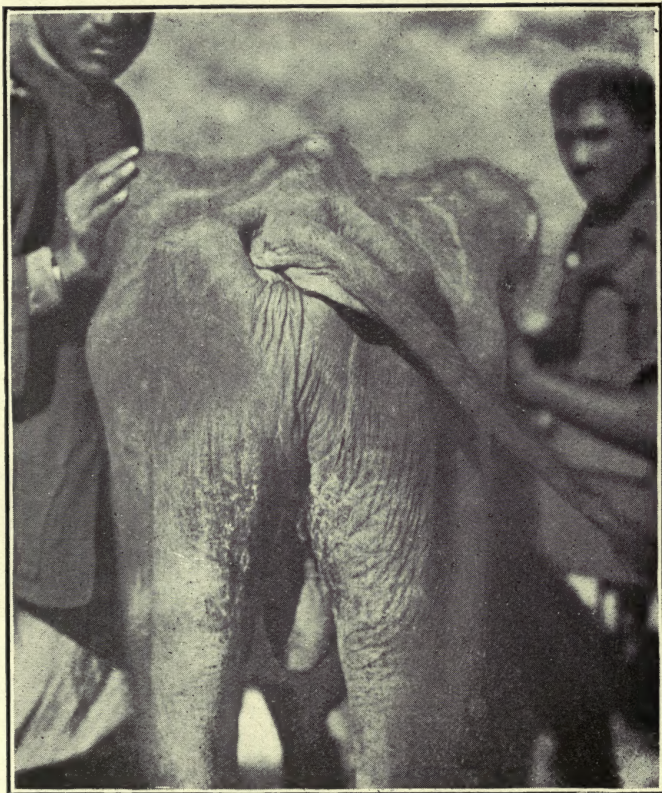
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Mange Parasites on perineal region and upper part of thigh of buffalo.

28.—THE BIONOMICS OF THE SARCOPTIC MANGE PARASITE
OF THE BUFFALO, WITH SOME OBSERVATIONS CONCERN-
ING THE RELATIVE POWER OF RESISTANCE TO ADVERSE
CONDITIONS OF THE DIFFERENT STAGES OF THE ACARUS
AND OF ITS EGG.

By T. M. TIMONEY, M.R.C.V.S., *Third Bacteriologist, Muktesar.*

(Plates 11—14.)

INTRODUCTION.

The two common forms of parasitic mange, *viz.*, the *sarcoptic* and the *psoroptic*, attack large numbers of buffaloes used in Muktesar for serum production during eight or nine months of the year. From May till August the disease disappears; but towards the end of the latter month it makes its reappearance simultaneously in different parts of the station. Since March of last year I was engaged in investigations relating to the sarcoptic *Acarus*, and except in the course of the few months already referred to, abundant mangy material could be easily procured at any time. A few weeks before the end of the monsoon season, the disease, which had been in a state of abeyance for at least three months, again became manifest in buffaloes which were accommodated in *kraals* widely separated from each other. The outbreak in each *kraal* was apparently spontaneous.

In scrapings taken from large numbers of buffaloes it was usual to find both species of parasites; most frequently the sarcoptic parasite was predominant, but cases occurred in which psoroptes were the prevailing species. About ten per cent. of the total cases displayed infection with sarcopts only; but no instance of a pure psoroptic infection came under my notice. The range of movement of the latter is much more restricted than that of the former. In this respect both parasitic forms correspond with their equine counterparts. Each form shows a predilection for the perineal region and the upper thigh (Plate 11). On the entire surface of the neck they frequently occur together (Plate 12), although in this situation the sarcopt is more often found than the psoropt. Other situations, such as the root of the tail, the poll, and the flanks, are comparatively infrequently involved in the disease, but, when these locations are attacked, the sarcopt is invariably the causative parasite. As the investigations which have occupied my attention during the past

nine months relate to three distinct phases in connection with the parasite, I propose to describe the results of my observations of each phase under a separate heading or part :—

Part I. A method is described which was found most advantageous for observing the daily average number of eggs laid by numerous ovigerous females in their galleries until the time of their death, and for studying the life-cycle of the Acarus.

Part II. The important problem dealing with the resistance of the parasite to adverse natural and artificial conditions is here studied. For convenience, I have divided this part into two sections. The first section deals with the longevity of each stage of the parasite apart from its host and exposed to the variable influences to which it may be subjected in its natural habitat. The results of numerous experiments upon the effects of sunshine, darkness, moisture, and dryness, operating at room temperature, are recorded. In the second section, the relative power of resistance of the different stages to the action of an efficacious acaricidal agent, viz., ten per cent. solution of creosote in olive oil, is considered.

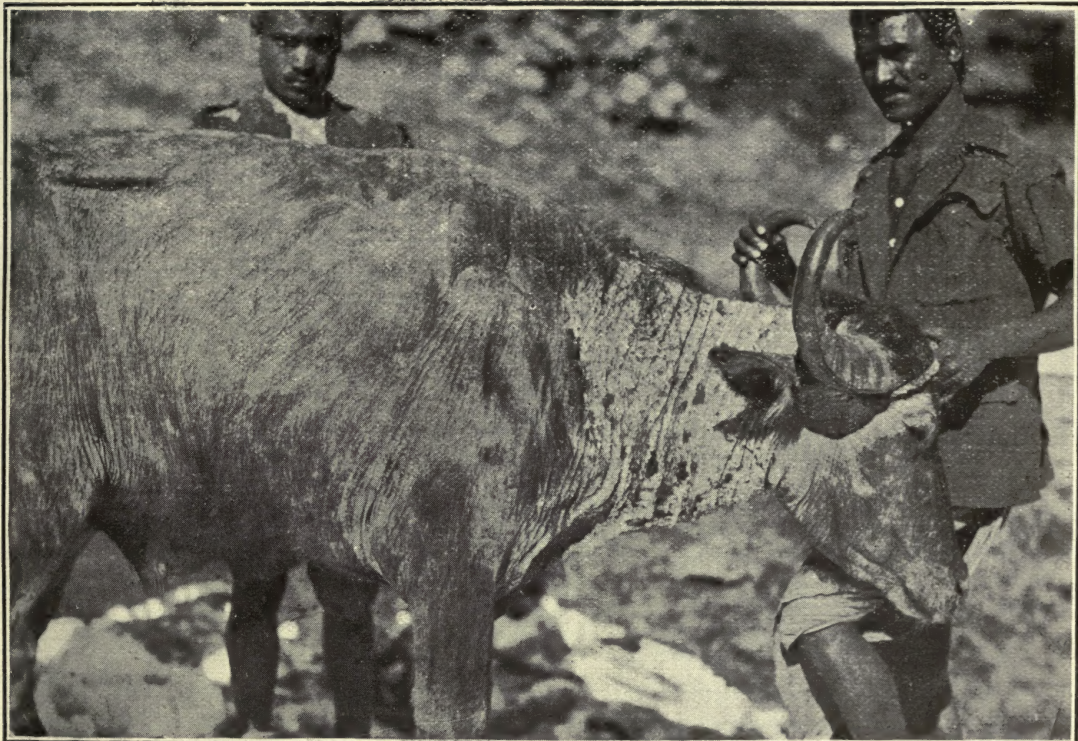
Part III. This Part is devoted entirely to the consideration of the resistance of eggs to exactly the same passive and active conditions as those studied in *Part II.* It is also divided into two sections; the first refers to the length of time an egg may be separated from the host on which it was laid without losing its faculty of hatching; and the second section deals with the effect of exposures of varying duration to a solution of ten per cent. creosote in olive oil on its vitality.

PART I.

Observations upon the prolificness of an ovigerous female, and the life-cycle of the sarcoptic parasite of the buffalo.

In order to carry out these investigations I imitated the method employed by Shilston* for studying the life-cycle of the *Psoroptes communis* var. *ovis* in South Africa. For his work, Shilston used glass tubes three inches deep, two inches in diameter, and one eighth of an inch in thickness. After he had prepared on the back of a sheep, by shaving and cleaning, a circular patch of skin of the same diameter as the tube, he fixed one end of a tube to the integument by running melted paraffin wax around its base and tied tufts of wool over the upper end. The wax and the strands of wool prevented the dislodgment of the tubes.

* SHILSTON, A. W. Sheep Scab. Some observations on the life-history of *Psoroptes communis* var. *ovis* and some points connected with the epizootiology of the disease in South Africa. *Veterinary Research Laboratory, Allerton, Pietermaritzburg, 1915.*



Mange Parasites on neck of buffalo. Both sides of the neck were equally affected.

For my experiments it was necessary to devise a means of fixing a circular glass cover to the back of a buffalo without supplementary aid. After testing the adhesive properties of a large number of substances, including paraffin wax, I had recourse to a special substance known commercially as "Chatterton's compound."* Instead of glass tubes of the kind Shilston used, I utilized a Petri dish cover with thicker walls. As the compound is obtainable in sticks, a small piece was chipped off with a knife and melted in an enamel basin: melting point 38°C. The fluid mixture was applied to the rim of the glass with a camel-hair brush. It may be added that, as the adhesive powers of this substance are small when it is dry and hard, it is essential that the glass circle should be placed on the animal immediately after the mixture has been applied to it. But, if the substance should have hardened, it may be melted again in a flame.

With experience, I learned that my experiments were vitiated when the rim of vertical glass wall was smeared over its entire thickness with the compound, as the substance becomes softened by the heat of the body and extends into the circular area circumscribed by the glass ring. Wandering Acari were trapped in it, and as they were unable to extricate themselves from it, they died quickly. Accidents of this nature were eventually obviated by applying the substance to the outer edge of the rim only.

Petri dishes of small size were used, the diameter ranging from inch to one and a half inches. The dishes must be small for two reasons: firstly, because the compound will only keep the dish fixed to the skin when its rim is resting on a flat surface; and secondly, it is impossible to find a flat area of greater width than one and a half inches on the body of even the largest buffaloes. If a rim is applied to an uneven surface the lack of contact at the low lying parts of the skin causes the occurrence of gaps through which the Acari can escape. The roof of each dish was perforated with a large hole for ventilation. The hole was made by allowing hydrofluoric acid to act upon an area of the glass of the desired dimensions for three or four days. Lastly, the hole in the dish was covered with a single layer of muslin, dyed black, and fixed with seccotine. The object of using muslin to cover the opening was to limit to a minimum the ingress of dust which would otherwise have entered the circumscribed area in abundance, and thus have added considerably to the difficulty of recovering eggs. As the Acari seldom dis-

* The formula and method of preparation of Chatterton's compound are as follows:—Stockholm tar one part; resin one part; pure gutta-percha three parts (by weight). Prepare by melting the tar and the resin in steam-jacketed vessels, and, after filtering the gutta-percha, add in thin shreds and mechanically mix by horizontal stirrers revolving in a vertical shaft.

played a tendency to climb up the surface of the glass and make their escape, loss of parasites through the opening was not much feared; but nevertheless, I deemed it expedient to dye the muslin black so as better to detect any that might have sought to escape. On a few occasions I have found Acari dead in the seccotine around the margin of the opening.

On account of the relative flatness of its surface, the portion of skin lying laterally between the vertebral column and the angle of the haunch was selected for my experiments. An ovigerous female that had been picked out of a scraping taken an hour or two previously was transferred to the flattest part of this area on the tip of a needle mounted in a wooden handle. A Petri dish unsmeared with the compound was placed over the area where the observation was intended to be made. The behaviour of the Acarus on finding itself in its natural habitat depended largely on the warmth of the day, and was especially influenced by sunshine. On a warm, sunshiny day, it became active immediately; but on a day when no sunshine gleamed on the skin its movements were sluggish for many minutes. In the majority of my observations the parasite wandered over the skin attempting to insinuate itself beneath the rim of the glass, but rarely attempted to climb up the ring. After a duration varying from ten to thirty minutes, it became stationary, and, in about ninety per cent. of my observations, the resting place was the root of a hair. The parasite remained in a state of immobility for quite a long time, the only movement discernible through a hand-lens being a vigorous activity of the anterior legs in the act of beginning excavation operations. This movement impressed me by its similarity to the movements of a dog tearing a depression in a sandy soil. By slow degrees the visible portion of the Acarus became less and less manifest, until finally the long bristles of the posterior legs disappeared from view. Neumann* states that the length of time occupied by an ovigerous female in excavating a gallery is from fifteen to thirty minutes; but I have repeatedly witnessed the female spending an hour and even longer, in tunnelling the initial portion of her gallery.

One female only was used for each observation. In quite a large number of cases it made no attempt to dig its way beneath the epidermis and was found dead the following day on the surface of the skin. As soon as the parasite was seen to disappear, the spot where the disappearance occurred was marked with a yellow grease pencil and the Petri dish fixed firmly above it. The next day the dish was removed and

* NEUMANN. Parasites and Parasitic Diseases of domesticated animals. Fleming Translation. London, 1892.

search made for the mite. By means of a needle, it was easy to lay open the track and discover it. The number of eggs was counted, and either removed or transferred to a fresh part of the enclosed field. The female was taken up and placed on a slide on the warming-stage to ascertain whether it was still alive and whether its movements were active or sluggish. It was replaced on the spot from which it had been removed and the observation continued. The removal of the parasite each day did not seem to hinder its egg-laying activities. Thirty observations of excavating females were recorded. After one day death took place in three cases; after two days in one; after three days in two; after four days in three; after five days in six; after six days in five; after seven days in four; after eight days in three; after nine days in two; after ten days in one. Ten days was the maximum duration of the life of an ovigerous female in experimental conditions. In most of the observations the female continued laying until the time of its death. Below is a table representing the aggregate number of eggs laid by the six females, three of which survived for eight days, two for nine days, and one for ten days.

TABLE 1.

Aggregate number of eggs laid by six females during ten days.

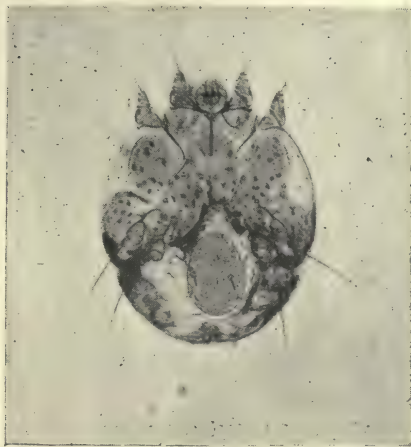
No. of days.	No. of eggs recovered.	Average for each female.	REMARKS.
1	Thirteen . .	Two and a half . .	One female contributed no eggs.
2	Nine . .	One and a half . .	All laying.
3	Ten . .	Nearly two . .	Do.
4	Five . .	Nearly one . .	Only four contributed.
5	Twelve . .	Two . .	Each laid two eggs.
6	Twenty . .	More than three . .	All laying.
7	Twenty-one . .	Three and a half . .	Do.
8	Fifteen . .	Two and a half . .	Do.
9	Six . .	Two . .	Only three laying.
10	One . .	One . .	Only one laying.

The total number of eggs laid during the first eight days, when all the mites were laying, was 105, *i.e.*, an average of seventeen to eighteen eggs for each female. This computation is not divergent from that made by Gerlach * whose calculations assigned fifteen eggs to each ovigerous female of *Sarcoptes scabiei* var. *hominis*.

* GERLACH, KRATZE AND RAUDE. Berlin, 1857.

Life-cycle of the buffalo Sarcopt.

Difficulties of an apparently insurmountable nature militated against achieving results of absolutely unquestionable significance regarding the life-cycle of this parasite. The hide of every buffalo is traversed by a net work of fine intersecting fissures, where the small forms of the larvæ and nymphs can effectually conceal themselves. In spite of every care it was found impossible to keep a male under observation for longer than half-an-hour on account of its dark-brown colour blending with that of the hide. Realizing that I could not hope to observe with indisputable accuracy the duration of each stage and the length of the quiescence synchronous with each moult, I determined to ascertain the number of days that elapsed between the hatching of the egg and the attainment of adolescence. A number of experiments was conducted with newly-laid eggs. It was easy to ascertain the interval between laying and hatching. In ninety per cent. of my observations the interval was two days. In very few cases three days were required for the development of the larvæ, and in a fairly large number of trials the interval was about one and a half days. A number of newly-laid eggs was put under cover, and two days allowed for them to hatch. At the end of three, four, five, six, seven and eight days, the covers were removed and a search made for 'the young forms.' On the day following upon the hatching of the eggs, *i.e.*, on the first day, one or two larvæ were occasionally seen. Usually they were careering across the area, and more rarely were lying motionless in a fissure or under a crust. When removed to the warming-stage they were extremely active. Sometimes a dead larva was recovered on this day. On the second day the few larvæ seen were distinctly larger than those seen on the previous day. They also were agile. On the third day, in a few of the numerous experiments I conducted, a small nymph was found. Larvæ could also be found in the fissures. On the fourth day no larvæ were ever met with; one or two nymphs of different sizes were occasionally picked up. On the fifth and sixth day the task of recovering the parasites was considerably easier than on any previous day, owing to the larger dimensions of the parasite, and the glistening whiteness of its body. The sixth day sometimes revealed nymphs of large magnitude, which might be considered pubescent. On the seventh day, only an isolated nymph of small size could be recognised; the majority of the parasites had reached the state of puberty. By the eighth day no nymph was ever recognised; all the mites had reached adolescence. When kept under cover no pubescent female survived longer than four days. Attempts to bring about the fertilization of the pubescents failed. On numerous occasions males and pubescents were



1. Ovigerous female, with egg.



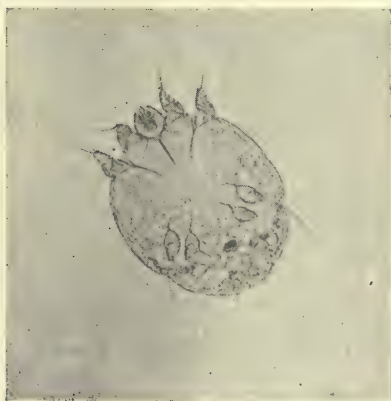
4. Male.



2. Pubescent female.



5. Large larva.

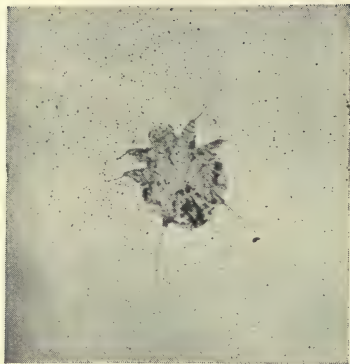


3. Nymph.

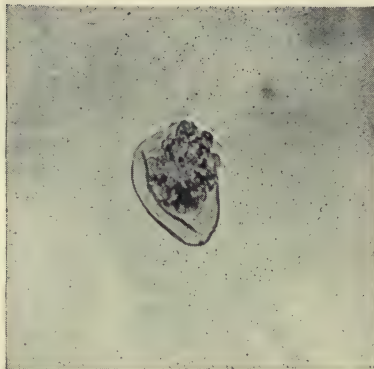


6. Small larva.

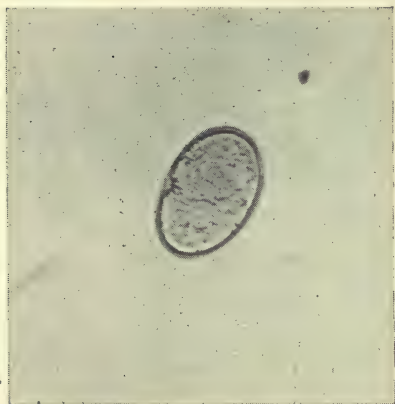
The various stages of the Sarcoptic Mange Parasite of the Buffalo.
(All figures magnified 41 diameters.)



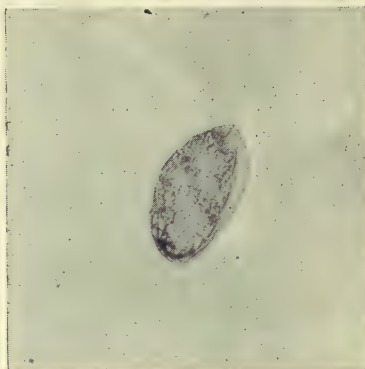
7. Newly-hatched larva.



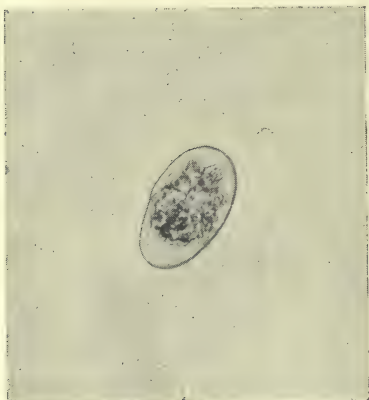
10. Larva emerging from egg.



8. Newly-laid egg.



11. Degenerated appearance of egg kept for five days at room temperature.



9. Larva inside egg-case.



12. Degenerated appearance of egg exposed for twenty minutes to ten per cent. creosote in olive oil.

placed together, but no evidence of copulation having taken place was ever discernible. These observations would therefore assign six to eight days as the interval between hatching and adolescence.

It may be appropriately mentioned here that the Sarcopt of the buffalo is considerably smaller than its equine counterpart. In the table below the comparative dimensions of the egg and the various stages or the two species are recorded. The measurements of the Sarcopt of the horse are from Mégnin, quoted by Noel Pillers.*

TABLE II.

Comparison between the dimensions of the Sarcopt of the horse and of the buffalo.

	SARCOPT OF HORSE.		SARCOPT OF BUFFALOES.	
	Length in m.m.	Breadth in m.m.	Length in m.m.	Breadth in m.m.
Egg	.16	.10	.12—.19	.10—.12
Larva	.16—.25	.10—.17	.15—.18	.10—.15
Nymph	.30	.20	.19—.23	.15—.19
Male	.26—.28	.18—.20	.19—.28	.16—.21
Pubescent	.35—.40	.25—.30	.27—.37	.19—.30
Ovigerous Female	.45—.47	.35	.36—.39	.28—.31

PART II.

SECTION 1. *The duration of vitality of the different stages of the Acarus apart from the body of their host, under varying conditions, in regard to temperature, light and moisture.*

For each experiment one ovigerous female, one pubescent female, two males, one nymph, and two larvæ were used. The mites were picked up with a needle mounted in a wooden handle, from a scraping in a Petri dish resting on the warming stage. At the beginning of the experiments different forms were recognized through the low-power objective of the microscope; but with the acquisition of a little experience, I was able to identify the stage of the parasite through a hand-lens magnifying ten times. The group of parasites was placed on an ordinary glass slide in the middle of a circle made with a yellow grease pencil. I assured myself that none of them had been injured in the course of being picked up by warming the slide before submitting the group to the longevity

* PILLERS, A. W. NOEL. "Notes on mange and allied mites" 1921.

test. Four groups were assembled; the first was placed on the bench of the laboratory in a place exposed to sunshine for two or three hours each day. The temperature in the sunlit part of the bench ranged from 16°C to 20°C; at other times of the day it varied from 10°C to 12°C. The second group was placed on the top of an incubator regulated at 31°C. The temperature of this situation was never lower than 13°C nor higher than 15°C. The sunbeams never reached the part of the room where the incubator stood. The third group was placed in a cupboard kept in total darkness, but effectually ventilated. The temperature of the cupboard ranged from 8°C to 10°C. The last group was placed in a situation inaccessible to the rays of the sun, and moistened with pieces of cotton-wool and cloth soaked in water. The temperature usually ranged from 7°C to 10°C.

At intervals of twenty-four hours each group was warmed on the stage, and replaced in its situation until all the members were dead. At least forty tests under the four conditions enumerated were carried out. The variations in the results of the tests on each group were remarkably slight. A typical result in each of the four groups is detailed below.

GROUP I.

(PLACED ON BENCH AND WARMED FOR TWO TO THREE HOURS DAILY BY THE SUN'S RAYS.)

After one day	All actively alive.
„ two days	One male sluggish; others active.
„ three days	One male motionless; the other male sluggish; both larvæ sluggish; nymph's vitality weakened; ovigerous and pubescent active.
„ four days	Both males and both larvæ motionless; nymph feeble; ovigerous and pubescent moving fairly actively.
„ five days	Nymph motionless; pubescent sluggish; ovigerous slightly weakened.
„ six days	Movements of pubescent very faint; ovigerous sluggish.
„ seven days	All motionless.

GROUP II.

(PLACED ON TOP OF INCUBATOR; INACCESSIBLE TO THE SUN.)

After one day	All actively alive.
„ two days	Both males and one larva feeble; others still active.
„ three days	Both males and both larvæ motionless. Nymph sluggish. Ovigerous and pubescent fairly active.
„ four days	All except ovigerous and pubescent motionless. Pubescent faintly alive. Vitality of ovigerous weakened.
„ five days	All motionless.

GROUP III.

(PLACED IN DARKNESS.)

After one day . . .	One male motionless. Both larvæ and the other male sluggish. Others active.
„ two days . . .	One larva motionless. The remaining larva and male dying; nymph only feebly alive; pubescent and ovigerous sluggish.
„ three days . . .	Both males and larvæ apparently dead. Nymph dying. Ovigerous and pubescent move with difficulty.
„ four days . . .	All motionless.

GROUP IV.

(PLACED IN MOIST, DIMLY-LIGHTED SITUATION.)

After one day . . .	One male and one larva motionless; the other male sluggish, all others active.
„ two days . . .	Both males and one larva motionless; the other larva dying; nymph sluggish; ovigerous and pubescent fairly active.
„ three days . . .	All except ovigerous and pubescent apparently dead. Both of the latter in a feeble state.
„ four days . . .	All motionless.

The results of the above experiments demonstrate with convincing clearness that the vitality of the male and the larva is about equal, and that the vitality of these two stages is much less than that of the other stages. In no instance did a male give evidence of possessing a greater vitality than that of an ovigerous female, and a larva lived as long as the latter on one or two occasions only. In all the experiments, a larva of large and small size were used. The former was invariably more resistant to the adverse conditions imposed upon it than was the latter. The vitality of the ovigerous female is just appreciably greater than that of the pubescent female. In quite a large proportion of the tests the pubescent survived the other, but the aggregate result of the experiments proves that the ovigerous female possesses greater powers of resistance. The position of the nymph in this respect is intermediate between that occupied by the male and the pubescent female. Not infrequently it lived as long as any other member of its group, but, in general, its resistance might be assigned a place inferior to that of the adults.

The effect of protracted exposure to cold on the vitality of the Acari is noteworthy. For a period of ten days or so at the beginning of last December, a wave of intense cold passed over Muktesar. On entering my laboratory in the morning, I observed the temperature of my working-bench to be 4°C or 5°C. The temperature did not rise above 7°C during the whole day except in places near the fire.

The group on the slide were dealt with in a manner precisely like that employed with Group I. The effect of sunlight was more than neutralized by the severity, and especially by the protracted duration of the exposure to cold. After two days both males and both larvæ were usually dead. The vitality of the others was noticeably reduced, and, in some observations, the nymph was in a dying state. On the following day the ovigerous and pubescent females alone retained indications of feeble vitality. After four days all were dead. About ten experiments were made during this cold weather, and only in one instance did an ovigerous female survive four days' exposure.

It is reasonable to assume that parasites left among the epidermal debris would survive for a longer period than those removed from it. Gerlach estimated the duration of the human Sarcopt when put in a watch-glass and exposed to ordinary summer temperature at five to six days; and the duration among scrapings at eight to ten days. The crusts had the property of prolonging life two-fold. My experiences differ entirely from those quoted. It was rare to note evidences of life in a scraping after the group of parasites which had been removed from it had died. On the contrary, I have frequently failed to notice in such circumstances a single living form in a scraping while the females on the slide were still distinctly alive.

PART II.

SECTION 2. *Relative degrees of resistance of the different stages to a solution of ten per cent. creosote in olive oil warmed to 31°C.*

The second series of tests was performed with a mixture of ten per cent. creosote in olive oil. Pure creosote was specially obtained for these tests on account of the unsuitability of the crude creosote already in stock. A member of each stage of the parasite was submitted to the action of this drug maintained in the incubator to 31°C, for varying periods of exposure.

The procedure employed was as follows. The scraping was taken in a paper envelope from a badly infected buffalo at the animal hospital, which is about a mile from the laboratory. On receiving the envelope, I divided its contents into two portions, which were put into separate Petri dishes. One dish was placed in the incubator at 31°C to be thoroughly warmed for half-an-hour before use, and the other was left on the bench. The temperature of the room was usually 12°C to 15°C.

The warming-stage was heated by means of a spirit lamp, and when the temperature of its surface reached 30°C to 32°C, the spirit lamp was

manipulated in such a manner as to ensure the maintenance of this degree of heat uniformly for several hours. Workers unaccustomed to the use of a warming-stage may be led into serious errors through lack of knowledge concerning the method of obtaining a uniform temperature throughout the platform. The thermometer incorporated with the stage registers a rise in temperature very slowly : when the mercury reaches the mark 30, it will be found that the temperature of the surface has ascended to 45° or higher. By keeping the bulb of a thermometer on the surface of the stage, I have observed that this part of the apparatus reaches 30°C, before the integrant thermometer records 20°C. With continued heating the difference between the actual and registered temperatures gradually decreases, until after half-an-hour it is less than two degrees. Ignorance of this important point was the cause of the vitiation of many of my earlier experiments. After the surface had remained uniformly at 30°C for a few minutes, the scraping was removed from the incubator and placed upon the stage. Meanwhile a number of slides containing each a large drop of the reagent was put into the incubator to be warmed to the required temperature. As soon as an ovigerous female, a pubescent female, a nymph, a male, and two larvæ (one large and one small) had been isolated from the scraping, a slide was taken from the incubator and put on the warming-stage. In order to make certain that all the Acari possessed complete vitality, the slide on which they lay was also placed on the stage at the side of the other. Almost immediately they began to run swiftly along the glass surface. They were then picked up one by one by means of a needle and placed in the drug. Any parasites that appeared to be injured were not used. The greatest activity was usually exhibited by the males and larvæ, which sometimes ran with great speed. The ovigerous female was always more sluggish than a member of any other stage. As the relatively greater resistance of the ovigerous and pubescent females to the action of the drug had been demonstrated beforehand in very numerous experiments, these stages were always placed in the drug first. The entire length of time occupied in transferring the six Acari never exceeded thirty seconds. The reason why they were placed in the same drop was that all of them could thus be observed at the same time with the low power lens. The duration of exposure ranged from three minutes to thirty minutes. At the expiration of the period of exposure, the parasites were rapidly washed with a reagent capable of removing oil quickly. After experimenting with a number of reagents, including xylol, ether, one per cent. acid alcohol, a weak solution of sodium hydroxide and warm water, I selected ether as the most rapid cleansing agent. A few drops of it were placed around the large drop of oil and allowed

to mix with it thoroughly. By tilting the slide the oil was run down its surface and wiped off with a piece of muslin cloth. A second application of ether was generally sufficient to wash completely away all traces of the oil. During this operation great care had always to be taken to avoid losing the mites. As the ether had now replaced the oil, and formed small pools about the bodies of the Acari, it was considered desirable to remove them to a part of the slide untouched with either oil or ether. The removal was effected by means of a special brush, because the slippery nature of their bodies resulting from contact with oil rendered them liable to be damaged in attempts to pick them up with a needle. The whole operation of washing and removing to a dry part of the slide usually occupied one minute. The slide was then replaced on the warming-stage to see whether any of the Acari revealed signs of reviving life or not. Only on rare occasions was evidence of resuscitation observed so soon after washing. The slide was next replaced in the incubator for one or two hours in order to accustom surviving parasites to favourable thermal conditions. At the end of this interval the slide was again placed on the warming-stage, and after inspection it was put on the laboratory bench. At various times during the ensuing three or four days it was warmed to ascertain whether the mites had revived from the effects of the drug, and, if they revived, how long life would still linger in them.

A few typical results of tests applied over a period of more than six months are given below.

Experiment 1.

<i>1 ovigerous, 1 pubescent, 1 male, 1 nymph, 2 larvæ (large and small) exposure</i>				3 minutes.
All struggled violently as soon as they found themselves in a harmful medium.				
<i>Male and small larva become quiescent after</i>				1½ minutes.
<i>Nymph and large larva</i>				2 "
<i>Ovigerous and pubescent</i>				2½ "
<i>After washing</i> . All motionless.				
1 hour later . All revive.				
4	"	"	"	"
24	"	"	"	Ovigerous, pubescent, nymph, male, and large larva alive.
48	"	"	"	Ovigerous and pubescent alive.
72	"	"	"	" " "
96	"	"	"	" " "
120	"	"	"	alive.
144	"	"	"	All dead.

In many experiments I used two males in order to provide against a possible accident to one.

Experiment 2.

1 ovigerous, 1 pubescent, 1 nymph, 2 males, 2 larvæ (large and small)	5 minutes.
Both males and small larva assume quiescence after	1 minute.
Large larva assumes quiescence after	1½ minutes.
Nymph and pubescent assume quiescence after	2 "
Ovigerous exhibits feeble movements up to	3 "
After washing	All motionless.
1 hour later	Ovigerous and one male revive.
3 hours later	All revive.
24 " "	Ovigerous, pubescent, nymph, one male, and large larva alive.
48 " "	Ovigerous and pubescent alive.
72 " " "	" " "
96 " " "	" alive.
124 " " "	All dead.

Experiment 3.

1 ovigerous, 1 pubescent, 1 nymph, 2 males, 2 larvæ (large and small)	10 minutes.
One male and small larva cease struggling after	1 minute.
The other male, nymph and large larva cease struggling after	2 minutes.
Pubescent ceases struggling after	2½ "
Ovigerous ceases struggling after	3 "
After washing	All motionless.
1 hour later	" "
3 hours later	All revive (one male feebly).
24 " "	All alive except one male.
48 " "	Ovigerous, pubescent and large larva alive.
72 " "	All motionless.

Experiment 4.

1 ovigerous, 1 pubescent, 1 nymph, 2 males, 2 larvæ (large and small)	15 minutes.
Both males and both larvæ become quiescent after	1½ "
Nymph and ovigerous become quiescent after	2 "
Pubescent become quiescent after	2½ "
After washing	All motionless.
3 hours later	All revive except one male.
24 " "	Ovigerous, pubescent and large larva alive.
48 " "	Ovigerous and pubescent alive.
72 " "	All motionless.

Experiment 5.

1 ovigerous, 1 pubescent, 1 nymph, 2 males, 2 larvæ (large and small)	15 minutes.
All except ovigerous and pubescent become quiescent after	1½ "
Pubescent becomes quiescent after	2 "
Ovigerous becomes quiescent after	2½ "

After washing . . .	All motionless.
2 hours later . . .	" "
3 " " . . .	Pubescent revives faintly.
4 " " . . .	Ovigerous, pubescent, nymph and large larva alive.
24 " " . . .	Pubescent and nymph alive.
48 " " . . .	" alive.
72 " " . . .	" alive.
96 " " . . .	All motionless.

Experiment 6.

1 ovigerous, 1 pubescent, 1 nymph, 2 males, 2 larvæ (large and small)	20 minutes.
One male becomes quiescent after	1 minute.
The other male, nymph and both larvæ become quiescent after	1½ minutes.
Pubescent becomes quiescent after	2 "
Ovigerous becomes quiescent after	2½ "
After washing . . .	All motionless.
1 hour later . . . " "	
3 hours " . . . " "	
24 " " . . . " "	

Experiment 7.

1 ovigerous, 1 pubescent, 1 nymph, 2 males, 2 larvæ (large and small)	20 minutes.
Both males, nymph and small larva become quiescent after	1½ "
Large larva and pubescent become quiescent after	2 "
Ovigerous becomes quiescent after	2½ "
After washing . . .	All motionless.
1 hour later . . . " "	
3 hours " . . . Ovigerous and pubescent revive feebly.	
6 " " . . . " feebly alive.	
24 " " . . . All dead.	

Experiment 8.

1 ovigerous, 1 pubescent, 1 nymph, 2 males, 2 larvæ	30 minutes.
All except ovigerous and pubescent cease moving after	1½ "
Ovigerous and pubescent cease moving after	2½ "
After washing . . .	All motionless.
1 hour later . . . " "	
3 hours " . . . " "	
4 " " . . . " "	
24 " " . . . " "	

Experiment 9.

1 ovigerous, 1 pubescent, 1 nymph, 2 males, 2 larvæ	30 minutes.
Both males and small larva become quiescent after	1 minute.

<i>Large larva, nymph and pubescent</i> become quiescent after		2 minutes.
<i>Ovigerous</i> becomes quiescent after		2½ "
<i>After washing</i>		All motionless.
1 hour later	" "	
3 hours	" "	
6 "	" "	
24 "	" "	

The examples given above form an epitome of several hundreds of tests carried out during a period of six or seven months. Examination of the results reveals the existence of a great difference in the degree of resistance to the effect of the drug among the various stages of the buffalo *Sarcopt*. In all the examples quoted, and in about ninety-five per cent. of the total tests, the ovigerous and the pubescent females displayed the greatest degree of resistivity; the male and the small larvæ occupy, as it were, the opposite pole in this respect, and the nymph and large larvæ lie in an intermediate position. All forms usually revived within twenty-four hours after exposure for ten minutes. After fifteen minutes exposure it was not unusual for all to revive, but the male and the larvæ were so exhausted from their contact with the drug that they did not retain their vitality long.

It is evident from the experiments of Henry * that the *Sarcopt* of the buffalo has a greater degree of resistance to this drug than the *Psoropt* of the horse. This author fixes fifteen minutes as the maximum length of exposure requisite to prevent resuscitation. Another feature of importance respecting the results of his investigations and mine is that he considered the larva slightly more resistant than the other stages, whereas in my inquiries its vitality was invariably inferior to that of the adults and nymph. In a few cases the male and the large larvae survived for twenty-four hours after the test, but the vast majority of them succumbed within a few hours after washing. One or two isolated examples of the survival of the small larvae are recorded in my note-book.

When exposed for twenty minutes, all forms died in about seventy per cent. of the tests; in the remaining thirty per cent. the ovigerous female, and less frequently the pubescent, revived. The revival was so feeble, however, that no evidence of life was ever seen in them after twenty-four hours. An exposure of thirty minutes effectually kills all stages. When tests were carried out with drops of the drug previously warmed to 31°C, and kept constantly at that temperature on the warming-stage for half-an-hour, no instance of revival was encountered. Many of my earlier experiments demonstrated the capability of the older forms of the parasites to resist exposure for thirty minutes, but in the light of

* HENRY, A. Sur le pouvoir acaricide de quelques substances utilisées dans la gale des équidés. *Rec. méd. vet.*, Sept. 1921, Vol. 94, No. 18, pages 357—382.

knowledge gained from further experience, I ascribe these results to faulty technique.

A feature of importance regarding the appearance of the parasite in the oily medium, and its revival from the action of the reagent, may be recorded here. As soon as the parasite was placed in the warm creosoted oil, a peculiar rotatory movement of abdominal elements began. This motion persisted long after the struggles of the *Acarus* had subsided. I observed that in nearly all cases in which this agitation continued up to the end of the test, resuscitation took place; but, on the other hand, revival of mites in whose bodies the movement had ceased, occurred with great frequency. The rotating elements lie about the middle of the body, and maintain a rhythmical oscillation, which varies considerably in its rate of movement. After three minutes in the drug, the motion usually reaches the point of most marked acceleration, and tends to become slower as the exposure is prolonged. This feature was observed in large number of all the stages except the male. On re-warming the slide after removing the *Acari* from the agent, the earliest sign of latent life was frequently evinced by the commencement of this agitation. If it developed in speed and in volume, early revival of the mite invariably occurred. If it continued to rotate slowly and inertly, feeble resuscitation or continued inactivity usually followed. In the case of the male, signs of life were frequently displayed by the passing of bubbles of oil up and down the oesophagus, before visible movements of the legs and jaws became discernible.

PART III.

SECTION 1. *The viability of the eggs of the buffalo Sarcopt under adverse conditions.*

The viability of the eggs was investigated in the conditions wherein the relative powers of resistance of the different stages of the *Acarus* were determined. First of all, I satisfied myself with regard to the period of incubation. Eggs which had been laid since inspection of females taken from the body on the previous day were removed to a fresh portion of the body, and covered with a Petri dish. All these eggs hatched within forty-eight hours after transference to the fresh patch. The same constancy in hatching was not displayed by eggs obtained from a scraping. About twenty per cent. of the latter were sterile.

The rapidity of hatching would seem to depend upon variations of atmospheric temperature. In summer few eggs remained unhatched at the end of twenty-four hours; but in winter hatching usually occurred on the second day. Eggs collected from a scraping taken on the morning of the day on which they were used, revealed no difference in respect

to the incubative period. As the eggs collected from the experimental buffalo had been laid not longer than twenty hours, the period of incubation would, therefore, range from two to three days.

It is interesting to compare this calculation with those made by various observers whose computations are quoted by Neuman. The eggs of the equine Sarcopt were the subject of their investigations. Gerlach calculated the incubative period at three days; Fürstenberg at six to seven days; Bourguignon and Eichstadt at ten days; Burchart at five days; Megnin at one to two days. Some of these observations were made in an incubator heated to the temperature of the body. When a collection of eggs was placed in an incubator, I noted that either they hatched within three days or they degenerated. I have never observed an egg, which did not hatch in three days in the incubator, retain its power to hatch when transferred to the body of the host.

A long series of experiments was made with the object of acquiring definite knowledge regarding the maximum length of time the eggs can retain their faculty of hatching when kept in a watch-glass at room temperature (10°C — 13°C). Most of the eggs used for these experiments were obtained from scrapings; but a small proportion of them were recovered from the experimental area on the buffalo. The eggs were divided into batches of ten, and laid on the bench for a certain length of time. The duration of the tests ranged from one to ten days. Two batches were submitted to the same period of insulation, and then incubated, one on the body of the animal, and the other in the incubator. The following table indicates the results of these tests.

TABLE III

Illustrating the number of days eggs of the buffalo Sarcopt, per batches of ten, can retain their power for hatching apart from the host, and the relative incubative value of the host's body and a thermostator regulated at 25°C — 27°C .

No. of days.	Incubated on body.	Incubated in thermostator.
One	Nine	Seven.
Two	Eight	Four.
Three	Six	Three.
Four	Five	One.
Five	Two	None.
Six	One	"
Seven	None	"
Eight	"	"
Nine	"	"
Ten	"	"

Each of these experiments was repeated three times in some cases and four times in others. In no instance did an egg which had been separated from the host for five days hatch in the incubator; and no egg separated for seven days hatched on the body of the animal. Shilston and Stockman (*ibid*) ascertained that a separation of eight days was the maximum an egg of the sheep scab *Psoropt* could withstand and retain its faculty of hatching. The former author replaced the eggs on the back of a sheep, and the latter used an incubator. Gerlach (*ibid*), records the interesting case of an egg of the equine *Sarcopt* retaining its germinative faculty after keeping it apart from its host for four weeks.

Figure 11, Plate 14, illustrates a typical appearance presented by an egg which has failed to hatch in the incubator on the completion of five days retention, and figure 12, Plate 14, the appearance of an egg that has been in contact with ten per cent. creosote in olive oil for twenty minutes and subsequently incubated.

SECTION II.

The effect of exposures of varying durations to a solution of ten per cent. creosote in olive oil on the germinative faculty of the egg.

Parallel experiments were made in precisely the same manner as described in detail in Section I of this part. The durations of exposure to the drug were the same as the *Acari* underwent. A batch of ten eggs was used for each experiment. In the Table below the results of these observations are recorded.

TABLE IV

*Illustrating the effect of exposure to a solution of ten per cent. creosote in olive oil for varying periods on the buffalo *Sarcopt* egg and the relative incubative value of the host's body and a thermostatator.*

No. of experiment.	Duration of exposure.	Incubated on body.	Incubated in thermostatator regulated at 25°C—27°C.
1	Three minutes	Eight hatched; five in two days and three in three days.	Five hatched; four in two days and one in three days.
2	Five minutes	Six hatched; two in two days, three in three days, and one in four days.	Three hatched; all in three days.

No. of experiment.	Duration of exposure.	Incubated on body.	Incubated in thermostat regulated at 25°C—27°C.
3	Five minutes .	Five hatched ; one in two days and four in three days.	Three hatched ; all in three days.
4	Ten minutes .	Two hatched in three days.	One hatched in two days, and one in three days.
5	Ten minutes .	One hatched in three days.	Two hatched ; one in one and a half days, one in three days.
6	Fifteen minutes	One hatched in three days.	One hatched in three days.
7	Fifteen minutes	Two hatched in three days.	One hatched in three days.
8	Twenty minutes	None hatched . . .	None hatched.
9	Twenty minutes	One hatched in three days	One hatched in three days.
10	Thirty minutes	None hatched . . .	None hatched.
11	Thirty minutes	None hatched . . .	None hatched.

The experiments pertaining to the shorter exposures were repeated three times : all others four times. Except on a few occasions, an exposure of twenty minutes in the drug kept at the constant temperature of 31°C was sufficient to prevent hatching. Though the number of larvæ that issued or attempted to issue from the egg-case was small, a large number, moreover, was seen to struggle vigorously inside the egg without being able to break through the egg wall. Figure 10, Plate 14, illustrates a larva in the act of emerging from the egg-case. The egg had been subjected to an exposure of ten minutes, and placed in the incubator. It was never observed that an egg which had undergone an exposure of thirty minutes in the drug revealed evidence of progressive development.

Investigations conducted by Stockman and Berry * regarding the effect of a creosoted dip (the composition of the dip and the proportion of creosote are not stated) on the vitality of the egg of *Psoroptes communis* var. *ovis* afford an interesting contrast to the results recorded

* STOCKMAN, S. AND BERRY, A. H. *The Psoroptes communis ovis* : some observations on Ova and ovipositing. Jour. Com. Path. and Ther., 1913. Vol. XXVI, pages 45—50,

above. They made three observations with the dip on a large number of eggs. In the first observation 100 eggs were placed in the medium for one minute and incubated at 25°C — 27°C : none hatched. In the second, eighty eggs remained in contact with the acaricide for half-a minute. none hatched. 300 eggs were used for the third experiment, and after an exposure of half a minute only one egg developed a larva.

Summary.

The average number of eggs laid by an ovigerous female under experimental conditions was seventeen to eighteen.

Ten days was the maximum duration of life of an egg-laying female.

The interval between hatching and the attainment of adolescence was six to eight days.

The ovigerous and pubescent females can withstand a longer separation from their host than can the other stages. The power of resistance to insulation is weakest in the male and small larvæ. The nymph and large larvæ occupy an intermediate position.

The influence of sunshine in prolonging and the action of cold in curtailing the resisting powers of the Acari were convincingly demonstrated.

An exposure of twenty minutes to the action of a solution of ten per cent. creosote in olive oil, warmed to 31°C , killed all males, nymphs, larvæ, and the majority of ovigerous and pubescent females. Tested by this acaricidal agent, the powers of resistance of the different stages corroborate the finding in regard to their resistance to more insulation.

The incubative period is one to two days at summer temperature, and two to three days in winter.

No egg separated for five days from the host hatched in the incubator (25°C — 27°C); and after seven days at room temperature (10°C — 13°C) no egg hatched when subsequently placed in the host's body.

Contact with eleven per cent. creosote in olive oil for thirty minutes kills all eggs. Only a few survive an exposure of twenty minutes.

Mr. Fletcher.

I should like to ask Mr. Timoney, if eggs are killed by the application of creosote, whether in practical application contact can be made between creosote and eggs laid in the galleries on the host?

Mr. Timoney.

In practice it was rather difficult; in the laboratory I actually put the egg in oil, as it was necessary to know the effect of the acaricide upon all the stages of the mite.

Mr. Edwards.

Mr. Timoney's paper represents only the commencement of the study of the important parasites causing mange. The disease is widespread

but very little work has been done on the subject ; and most of the important observations on the bionomics of these parasites date back to the early half of last century. Some modern work has been done in South Africa and Europe. No improvement however in methods of control has been made since the time of the Greeks. During the late war many methods of treatment were tried but no exact knowledge of the life cycle was obtained. Henry in 1920 estimated the effect of drugs on mange parasites and found that most of the household remedies were ineffective. Thorough knowledge of the life-cycle may show up weak points at which the parasites may be attacked. Work will be continued until a satisfactory method of treatment has been discovered. Mr. Timoney's paper represents a considerable step forward in the knowledge of the life-history of the mange parasite.

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